Mitochondria still possess some features telling of their endosymbiontic origin like their own DNA and fission/fusion machineries. As the cell grows and increases its size, the need for energy feeds back via nuclear transcription regulation in order to upregulate genes involved in mitochondrial biogenesis [1]. As more than 99% of all mitochondrial proteins are encoded in the nucleus, they are translated on cytosolic ribosomes and post-translationally imported into the organelle. This process starts at an essential protein complex in the outer membrane of mitochondria, called Translocase of the Outer Membrane or TOM complex [2].

In a recent study [3] we identified several phosphorylated residues in this complex and found that mitochondria are much more subjected to cellular signalling cascades than previously thought [4,5]. The mitotic state of the cell is reflected in the activity of cyclin-dependent kinases (CDK), and indeed, at least one TOM protein has been identified as a target for this group.

This study now focuses on the role of CDK-dependent phosphorylation of Tom. We demonstrate that the yeast CDK phosphorylates Tom6 specifically during the M Phase of the cell cycle. This phosphorylation influences the TOM complex composition under conditions, when mitochondrial biogenesis needs to be stimulated.

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7P8

Adaptation of respiratory chain biogenesis to cytochrome *c* oxidase deficiency caused by *SURF1* gene mutations

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Mutations in *SURF1* gene leading to loss of an assembly protein Surf1 are frequent cause of severe defects of cytochrome *c* oxidase (COX). Clinical manifestation of these mutations is Leigh syndrome — a fatal neurodegenerative disease. Surf1 appears to be involved in an early step of COX assembly but its exact function remains unknown. In our study we focused on alteration of COX assembly process as a consequence of *SURF1* mutations, on differences in supercomplex (SC) formation due to decreased amount of COX, and how *SURF1* mutations influence protein and transcript levels of oxidative-phosphorylation system (OXPHOS) and other pro-mitochondrial genes. For experiments we used fibroblast cell lines from 9 patients with different *SURF1* gene mutations and control cells. Proteins solubilised from inner mitochondrial membrane with mild detergents were analyzed using blue native electrophoresis in combination with SDS PAGE and Western blot detection. Transcript levels were determined with Agilent 44 k human genome microarray technique.

Decreased COX level (to 30%) in patient's mitochondria was accompanied with upregulation of complexes I, III and V (130–150%) and accumulation of Cox5a subunit. Whole genome expression profiling showed general decrease of transcriptional activity in patient's cells and indicated that observed changes in OXPHOS complexes have to be due to posttranscriptional compensatory mechanisms.

Fully assembled and functional COX was present mainly in $I–III_2–IV$ SC in patient mitochondria, while in control mitochondria the content of COX in SCs was comparable to that of free COX monomer. Lack of COX in patients further led to accumulation of basic $I–III_2$ SC form, complex III dimer and COX assembly intermediates. Using 2D electrophoresis we identified two comigrating COX assembly intermediates in the 85–130 kDa region. One was the originally proposed S2 intermediate consisting of Cox1, 4, and 5a subunits and the other one contained large amount of Cox1 subunits. It could represent Cox1 associated with other unknown proteins but almost no Cox4 and Cox5a subunits. Both intermediates were completely unable to associate with complexes I and III into SCs [1].

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7P9

Two pentatricopeptide repeat proteins are essential for biogenesis of the NADH:ubiquinone oxidoreductase from the filamentous fungus *Neurospora crassa*

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The NADH:ubiquinone oxidoreductase is the first and largest complex of the mitochondrial respiratory chain. Its assembly involves subunits encoded by the mitochondrial as well as the nuclear genome and requires biogenesis factors, which are not part of the mature complex I. Pentatricopeptide repeat (PPR) proteins are known to be involved in various steps of gene expression in organelles [1]. Eight genes encoding PPR proteins are found in *Neurospora crassa* (*N. crassa*). We determined the relevance of these genes for the assembly of complex I by characterizing the corresponding knockout mutants obtained by a high-throughput programme [2]. The identification of complex I defects was accomplished by measuring the NADH:ferricyanide redox activity and Blue Native polyacrylamide gel electrophoresis. Two knockout mutants were specifically affected in the assembly of complex I. The presence of a peripheral arm and the absence of a detectable membrane arm were demonstrated. Assembly intermediates were detected by western blot analysis using specific polyclonal antibodies against different complex I subunits. Moreover, a specific influence of the PPR proteins on processing respectively on amounts of mitochondrial RNA was observed by means of Northern Blots. To characterize their specific impact on biogenesis of complex I, the two proteins were expressed heterologously in *Escherichia coli* (*E. coli*) and purified via immobilized metal ion affinity chromatography (IMAC) and size

exclusion chromatography (SEC). Interaction studies with the proteins and the specific mRNA and/or DNA *in vitro* for example via electrophoretic mobility shift analysis will follow.

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7P10

A mitochondrial reporter system for studies on NRF-1/AMPK activity and regulation in viable cells

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Mitochondrial biogenesis is subjected to complex physiological control and mitochondrial mass and capacity vary according to the cell specific demands for respiratory energy.

Abundance of mitochondria can be modulated in response to physiological conditions such as exercise, cold exposure, caloric restriction and oxidative stress, cell division and renewal, and differentiation

We have generated a mitochondrial targeted GFP reporter cell model system to be able to study mitochondrial biogenesis. The cells are stably expressing a reporter construct regulated by nuclear respiratory factor 1, NRF-1. This transcription factor is essential for transcription of genes contributing to the mitochondrial DNA transcription machinery and genes encoding components of the respiratory chain among others.

Containing a NRF-1 regulated and mitochondria targeted GFP construct, the cell model simultaneously reports transcriptional regulation of NRF-1 target genes, as well as parameters of mitochondrial mass and morphology.

The AMPK activator, AICAR, has been used to induce mitochondrial biogenesis in these cells and various molecular techniques have been used to study the effects of NRF-1 activation on mitochondrial biogenesis. Repeated stimulation and cell-sorting were employed to select populations presenting dynamic regulation of mitochondrial biogenesis.

Different known activators of mitochondrial biogenesis are currently being used to test the reporter system in both malignant and non-malignant cell lines.

This model system provides unique possibilities to identify new mechanisms regulating mitochondrial function.

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7P11

Cytochrome *c* oxidase assembly factor Surf1: Candidate for heme *a* insertion into subunit I

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Biogenesis of the mitochondrial cytochrome *c* oxidase (COX) is a highly complex process involving more than 30 known accessory proteins. Here we focus on the steps taken by heme *a* from its site of synthesis, heme *a* synthase (HAS), to its final target site in COX subunit I, using the soil bacterium *Paracoccus denitrificans* as model organism. Studies in humans have shown that the dysfunction of the Surf1 protein is associated with Leigh syndrome, exemplifying the crucial role of this protein in cytochrome *c* oxidase maturation. Its heme binding properties, which have been confirmed *via* isothermal titration calorimetry for both *Paracoccus* homologues (Surf1c and Surf1q) [1] imply a role as heme stuttle. Further *in vitro* and *in vivo* interaction studies between Surf1 and heme *a* synthase support the idea that Surf1 is responsible for the specific uptake of heme *a* from HAS, its sequestration and coordinated insertion into COX subunit I [2]. Our current focus is now on the detailed elucidation of the molecular interaction of heme *a* with Surf1 on the one hand and HAS/Surf1 on the other hand, employing X-ray crystallography and solid state NMR techniques.

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7P12

Mgr2 promotes coupling of the mitochondrial presequence translocase to partner complexes

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As most mitochondrial proteins are encoded in the nucleus and synthesized as precursors in the cytosol, they must be post-translationally imported into mitochondria. After passage of the outer mitochondrial membrane via the TOM complex (Translocase of the Outer Membrane), precursor proteins with N-terminal, cleavable presequences are passed on to the TIM23 complex (Translocase of Inner Membrane 23), which mediates their further sub-mitochondrial sorting: Percursors with an internal hydrophobic stop-transfer signal are laterally released into the inner membrane, whereas water-soluble precursors without such membrane-insertion-signals are translocated to the mitochondrial matrix with the help of an ATP-driven import motor.

We have employed a systematic proteomic approach to analyse the composition of the TIM23 complex and identified the inner membrane protein Mgr2 as a novel genuine subunit of this translocase. Mgr2 recruits the regulatory component Tim21 to the essential TIM23 core complex. The Mgr2/Tim21 module is required for the efficient coupling of respiratory chain supercomplexes to the TIM23 machinery. Association of TIM23 with the respiratory chain facilitates the membrane-potential-dependent step of precursor protein insertion into the inner mitochondrial membrane. Moreover, Mgr2 plays an important role in the hand-over of precursor proteins from the TOM to the TIM23 complex. Consequently, Mgr2-deficient